

# Ethanollic Extract of *Moringa stenopetala* Leaves Enhances the Quality Characteristics and Shelf-Life of Vacuum-Packed Pork Patty during Refrigeration Storage

Semeneh Seleshe, Ammara Ameer, Beom-Joon Kim, and Suk Nam Kang

Department of Animal Resource, Daegu University, Gyeongbuk 38453, Korea

**ABSTRACT:** Studies reported that the moringa plant provides various bioactive compounds. The present study investigated the effect of an ethanollic extract of *Moringa stenopetala* leaves (MLE) on the storage consistency of vacuum-packed pork patty during refrigeration storage (4°C). Four treatments prepared were: control (without any preservative) and patties blended with 0.1% MLE (MLE1), 0.05% MLE (MLE2), and 0.2% potassium sorbate (PS). Patties incorporated with 0.05 and 0.1% MLE scored significantly lower ( $P<0.05$ ) pH values compared with the control at the final storage time and thiobarbituric acid reactive values as compared to both the control and PS treated patties throughout the study period. Moreover, patties added with MLE had a significantly lower ( $P<0.05$ ) total plate count than the control and the count decreased concomitantly with an increase in MLE concentration. The addition of MLE (0.1 and 0.05%) presented significantly higher ( $P<0.05$ ) redness ( $a^*$ ) values than the control across storage time. Sensory attributes of samples did not vary significantly ( $P>0.05$ ), and all treatments had similar overall acceptability scores during storage. In conclusion, incorporation of 0.1% and 0.05% MLE suppressed microbial growth and delayed the onset of oxidative rancidity in pork patties during storage without any effect on the sensorial properties and overall acceptability. As an organic preservative, MLE can help in extending the shelf life of pork patties to satisfy the demands of modern consumers for organic, healthy, and safe food ingredients.

**Keywords:** ethanollic extract, pork patty, sensory attributes, vacuum-packed, *Moringa stenopetala* leaves

## INTRODUCTION

Meat and meat products are vulnerable to quality degradation due to their high nutritional content (Devatkal et al., 2014). Some of the important factors that influence the shelf life of meat and meat products are enzymatic changes, microbial spoilage, and oxidation of lipid/protein (Kanner, 1994). As a result of the trends in the consumption of fast and convenient foods, the production of ground meat and other processed meats increased dramatically in recent years. In processed meats, patty-like products produced from minced meat cause the surface area, free water level, and pH to increase as cellular integrity is disrupted during the size reduction. As a result, these reforms build a robust environment for the proliferation of spoilage and pathogenic microorganisms, as well as encouraging oxidative changes (Kim et al., 2013). The prevention of microbial contamination and oxidative peroxidation during the preparation and processing of

meat products, therefore, plays a crucial role for consumers and meat processors alike.

Synthetic preservatives, antimicrobials and antioxidants have been extensively utilized in the food industries to prevent or minimize degradation in the quality of meat products, thereby addressing quality and shelf life of these products. In the food industry, synthetic preservatives are intensively used as they're more effective and less costly than natural preservatives. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary-butyl hydroquinone, and propyl gallate are among synthetic antioxidants widely applied in meat and meat products (Biswas et al., 2004; Jayathilakan et al., 2007; Shah et al., 2014). However, because of their apparent toxicological concerns, the use of these synthetic antioxidants has been brought into question (Juntachote et al., 2006; Nuñez de Gonzalez et al., 2008; Shah et al., 2014). Similarly, it was claimed that the use of synthetic preservatives such as nitrites and nitrates could have a carcino-

Received 1 June 2021; Revised 27 July 2021; Accepted 12 August 2021; Published online 30 September 2021

Correspondence to Suk Nam Kang, Tel: +82-53-850-6726, E-mail: sk-kang@daegu.ac.kr

Author information: Semeneh Seleshe (Researcher), Ammara Ameer (Graduate Student), Beom-Joon Kim (Researcher), Suk Nam Kang (Professor)

Copyright © 2021 by The Korean Society of Food Science and Nutrition. All rights Reserved.

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

genic effect on the human body and compromise consumer health (Decker et al., 1995). In general, these synthetic chemicals are regarded as unhealthy both by health professionals and consumers (Tang et al., 2001).

In recent years, the use of natural preservatives, particularly those from plant sources, is receiving a considerable amount of attention due to growing consumer demand and health concerns. Plants are consistently the reliable source to supply human beings with important bioactive substances. It is recognized that several plants produce ranges of phytochemicals that are prospective sources of natural antioxidants and antimicrobials include polyphenols, phenolic acids, flavonoids, tannins, and carotenoids (Muthukumar et al., 2014). The use of plant extracts as antioxidants and antimicrobials have been intensively investigated in various meat products. These studies showed a significant improvement in respect to the application of plant extracts in meat products as a natural preservative. These antioxidants prevent the oxidation of lipids and meat pigment deterioration, thus helping to inhibit the onset of rancid flavors and maintain meat color. Spoilage arisen from microbial activity could be also prevented or retarded by the use of these extracts.

Moringa plants are among high-value trees belonging to the Moringaceae family which comprises 13 species (Padayachee and Bajinath, 2012). *Moringa oleifera* is indigenous to Northern India's sub-Himalayan tracts which is typically mentioned as "horseradish tree" or "drumstick tree" (Jahn, 1991). Whereas *Moringa stenopetala* is commonly recognized as the East African moringa tree, according to Abiyu et al. (2018), as it's native only to Southern Ethiopia and Northern Kenya. The moringa plant is a source for several bioactive compounds like isothiocyanates and glucosinolates and an exceptional combination of quercetin, zeatin, beta-sitosterol, kaempferol, and caffeoylquinic acid, as well as alkaloids such as moringinine and moringine in the stem bark (Fahey et al., 2001). Moringa leaves have also been attested to drastically extend the shelf life of foods, which may be due to the presence of different forms of natural antioxidant compounds, such as ascorbic acid, carotenoids, and phenolic substances (Siddhuraju and Becker, 2003). Among 13 species of *Moringa*, only *M. oleifera* has been given research and development consideration and there are limited documents in respect to the functional activity of *M. stenopetala* species and its uses as a natural food preservative. Consequently, the purpose of this study was to investigate the effect of ethanolic extract of *M. stenopetala* leaves (MLE) on the storage stability of vacuum-packed pork patty as compared with commercial inorganic preservative, potassium sorbate (PS), during refrigeration storage (4°C).

## MATERIALS AND METHODS

### Preparation of ethanolic extract of *M. stenopetala* (MS) leaves

Healthy and un-infected leaves of MS were gathered from Hosaena, the Southern part of Ethiopia. The leaves were thoroughly cleaned under running tap water to remove dust and other foreign particles, air-dried under the shed, and then ground into powder using mortar and pestle. The powders were packed in polythene bags and brought to the experimental site, Animal Resources Department, Daegu University. The process of extractions from MS leaves powder was performed using 70% ethanol. Fifteen grams of the leaves' powder were weighed, added into 500 mL conical flasks, and 150 mL of ethanol was poured into the flasks. The mixture was shaken regularly every 6 h and held for two days at room temperature (21 ~ 23°C). The extract was filtered by filter paper (Whatman No. 1, GE Healthcare, Buckinghamshire, UK) and concentrated at 45°C using an evaporator (EYELA CCA-1110, Tokyo Rikakikai Co., Tokyo, Japan) under reduced pressure. The extract was lyophilized and kept in a desiccator until use.

### Preparation of patties

Fresh pork loins (*longissimus dorsi* muscle) used for the study were purchased from Geyongsan meat market, Korea. After the connective tissues and excess fat were trimmed, the lean meat was kept in the refrigerator (4°C) for a day until it was used. Chilled pork samples and pork fat were cut into small cubes and minced twice using a meat mincer (M-12S, Hankook Fujee Industries Co., Ltd., Suwon, Korea). The basic patty formulation included lean pork meat (72%), pork fat (15%), water (ice) (10.37%), salt (1.10%), nitrite pickling salt (a mix of NaCl and nitrite, 97:3) (0.40%), sodium phosphate (0.15%), sugar (0.41%), glucose (0.43%), monosodium glutamate (L-glutamic acid) (0.08%), and ascorbic acid (0.06%). All ingredients were thoroughly blended using a bowl cutter (K15, Talsabell s.a., Valencia, Spain). Then, the batter was divided into four batches and randomly assigned into four different treatments (1 kg each). One lot was allocated as control (without any preservative) and the other three lots were thoroughly blended with their respective treatments: 0.1% MLE (MLE1), 0.05% MLE (MLE2), and 0.2% PS using a rotary food mixer (Spar Food Machinery MFG Co., Ltd., Taichung, Taiwan). Patties (100 g) were molded using a patty former. Then, patties were cooked in a digital Hi-cook chamber unit (SMK-2000SL, Metatek Co., Ltd., Nonsan, Korea). Each batch was aligned in different manufacturer-supplied trays, and a thermostat attached with the regulating system was inserted in the core/center of the patty for detecting the temperature during the cooking experiment. Cooking of the samples was performed at 70°C internal temperature for 15 min.

The weight of raw and cooked samples was recorded to calculate the percentage of cooking yield. Patties were packed in Nylon/PE bags (Gasung Pak Co., Ltd., Gyunggi, Korea) under vacuum (Model 19/S, Röscherwerke GmbH, Hanover, Germany) and kept in a refrigerator at 4°C for 30 days. Storage studies of samples were conducted at 5, 15, and 30 days of storage.

#### Cooking yield

Cooking yield was calculated by recording the weight of patties before and after cooking. The percent of cooking yield was calculated as follows:

$$\text{Cooking yield (\%)} = \frac{\text{Weight of the cooked patties}}{\text{Weight of raw patties}} \times 100$$

#### Analysis of pH

The pH values of patty samples were analyzed using a digital pH meter (Mettler Toledo, Columbus, OH, USA). Three grams of sample was homogenized with 30 mL of distilled water for 1 min using a homogenizer (Model Polytron® PT 2500 E Stand Dispersion Device, Kinematica AG, Malters, Switzerland) and the pH value of the sample was recorded. Analysis of pH was performed in three replicates per each treatment and storage day.

#### Instrumental color analysis

Instrumental color analysis of patties was conducted using a Chroma meter (CR 300; Minolta Co., Osaka, Japan) with illuminant C, D65, diffuse illumination/0° viewing angle, 8 mm aperture size (illumination area) after calibrating with a manufacturer-supplied white calibration plate ( $Y=92.80$ ,  $x=0.3136$ , and  $y=0.3194$ ). Color reading was performed on the entire outer surface of the patties. Five spectral data were measured for each sample and the mean value for  $L^*$ ,  $a^*$ , and  $b^*$  was estimated from five random readings. Then, color values were expressed as lightness, redness, and yellowness from the respective scores of  $L^*$ ,  $a^*$ , and  $b^*$ , respectively, according to the CIE color system.

#### Microbial count

Microbial quality analysis of patties was conducted by enumeration of total plate count and yeast and mold. About 25 g portion of a sample from each patty was taken aseptically with a sterile spoon, mixed with 225 mL of autoclaved distilled water, and homogenized in a Stomacher Lab Blender (model 400 Circulator, Seward Laboratory Systems Inc., Suffolk County, NY, USA) for 1 min. Serial 10-fold dilutions ( $10^{-1}$  to  $10^{-7}$ ) were prepared by diluting 1 mL of the sample in 9 mL of autoclaved distilled water. Total plate count (TPC) was determined on plate count agar (Difco Laboratories Inc., Franklin Lakes,

NJ, USA), and yeast and mold on potato dextrose agar. Plates from different dilutions were incubated at 37°C for 48 h (Drosinos et al., 2005). The average numbers of colonies per countable plate were counted and total numbers of colonies per gram (CFU/g) were determined, and then presented as log CFU/g.

#### Thiobarbituric acid (TBA) reactive (TBARS) analysis

Analysis of lipid oxidation was performed by analyzing the TBARS value of patties during storage according to Pikul et al. (1989) with little modification. Briefly, the patty sample (5 g) was homogenized with 15 mL of distilled water and 50 µL of BHA (7.2% in ethanol) and then centrifuged at 2,000 rpm for 15 min using a centrifuge (Hanil Science Industrial, Co., Ltd., Incheon, Korea). The supernatant (2 mL) was mixed with 4 mL TBA solution (20 mM) in 15% trichloroacetic acid. The sample was cooled with the use of cold water after heating the mixture at 90°C for 30 min in a water bath. Therefore, TBARS were extracted from cooled samples. The absorbance of each sample was determined at 532 nm using a spectrophotometer (Multiskan Go, Thermo Fisher Scientific, Waltham, MA, USA). TBARS value was estimated as mg malonaldehyde/kg (mg MA/kg) of patty by multiplying odd ratio value with a K factor of 5.2.

#### Sensorial analysis

Sensory analysis was performed using descriptive sensory analysis (scoring method) for color, aroma, texture, flavor, juiciness, and overall acceptability attributes of patties. Seven trained panelists were involved in the sensory evaluation who are in the department of animal resources, Daegu University. Two weeks prior to the actual evaluation, panelists were trained in sensory quality attributes of the patty using commercial pork patty products. The panelists were given 3 slices of samples (20×10×10 mm) on white plastic dishes during the assessment. For all samples, codes with three different digits were given separately and served randomly to prevent carry-over results. Before each sample was evaluated, the panel was provided cold water to rinse their mouths. The panel made an evaluation out of a 5-point score. The score assigned to express the intensity of each described attribute was from 1 'lowest intense' to 5 'highest intense'. The sensory evaluation procedure was approved by the life management committee of Daegu University, IRB number (1040621-201905-HR-004-02).

#### Statistical analysis

The current experiment had 36 observations (four treatments×three batches×three storage periods). Statistical analyses of experimental data were performed using one-way ANOVA with SAS software version 9.4 (SAS Institute, Cary, NC, USA). For all evaluations, a significance

level of  $P < 0.05$  was used. Also, Duncan's multiple range tests were used to detect differences among mean within each factor.

## RESULTS AND DISCUSSION

Owing to the expected health benefits and efficacy of organic ingredients, interest in the utilization of natural antioxidants, and antimicrobials to improve the shelf life of meat products has increased noticeably in recent decades. The current study investigated the addition of 0.05 and 0.10% MLE as compared to the commercial inorganic preservative, PS, on the storage stability and quality characteristics of vacuum-packed pork patty during refrigeration storage ( $4^{\circ}\text{C}$ ). Incorporation of MLE at 0.05 and 0.10% concentration exhibited no substantial difference ( $P < 0.05$ ) on cooking yield percentage as compared to the control and PS treated pork patties (Table 1). The finding of the present study is comparable with previous study reports. Hazra et al. (2012) observed that, compared to the control, the addition of 1.0, 1.5, and 2.0% drumstick leaves to cooked ground buffalo meat revealed no substantial difference in cooking yield. Das et al. (2012) also reported that raw ground goat meat patties treated with 0.1% *M. oleifera* leaves (MOL) extract had no significant variation in cooking yield compared to control. Similarly, the research finding of Najeeb et al. (2015) demonstrated that incorporation of 1.0% powders of moringa, curry, mint, and BHT (200 ppm) in restructured chicken slices had no substantial difference in the cooking yield.

Determining the pH value is critically important as it has a profound effect on many characteristics of meat and meat products, including shelf life, color, and texture (Mahmoud et al., 2017). In the present study, all samples,

treated with MLE and PS, exhibited similarly lowered pH values than the control on the 5th day of storage. Thereafter, patties in MLE1 treatment scored prominently lower ( $P < 0.05$ ) pH values than others up to 15 days of storage. Moreover, samples in the MLE2 batch were comparable with the patties treated with the commercially used inorganic preservative, PS, across the storage days (Table 1). On the 30th day of storage, pH values of 6.45, 6.40, 6.43, and 6.43 were exhibited for control, MLE1, MLE2, and PS, respectively. Similarly, Shah et al. (2015) detected a marked change in pH of raw beef treated with MOL extracts in a modified atmosphere package. However, Muthukumar et al. (2014) reported no difference in pH among the control and treated samples in cooked pork patties due to the addition of a water extract from MOL as antioxidants. In all treatments of pork patties, there was an increase ( $P < 0.05$ ) in pH value as the storage time extended. During storage, the pH of products rose steadily and patties had a significant ( $P < 0.05$ ) higher scores on the 30th day of storage. The rise in pH may be accredited to microbial metabolites. The increases in pH may be due to the deposition of ammonia as a product of amino acid degradation, which was released during protein breakdown following the depletion of stored glucose by bacteria (Gill, 1983).

Oxidation of lipid is the major non-microbial cause for quality deterioration of meat products during storage and monitored by measuring TBARS (Falowo et al., 2014). The results for TBARS analysis of patties incorporated with MLE during the storage time are presented in Table 1. Treatments showed a significant variation in TBARS values across the storage time. In all storage days, MLE treated patty samples had significantly lower ( $P < 0.05$ ) TBARS values as compared to both the control and the commercial preservative, PS. Patty samples added with

**Table 1.** Cooking yield, pH, and TBARS values of patties incorporated with MLE during the storage time

Attributes	Days	Treatments of patties <sup>1)</sup>				SEM
		Control	MLE1	MLE2	PS	
Cooking yield pH	0	91.02	91.77	91.64	91.19	0.86
	5	6.40 <sup>aB</sup>	6.34 <sup>bB</sup>	6.35 <sup>bB</sup>	6.35 <sup>bB</sup>	0.01
	15	6.38 <sup>aB</sup>	6.34 <sup>bB</sup>	6.36 <sup>aB</sup>	6.37 <sup>aB</sup>	0.01
	30	6.45 <sup>aA</sup>	6.40 <sup>cA</sup>	6.43 <sup>bcA</sup>	6.43 <sup>bcA</sup>	0.01
	SEM	0.01	0.01	0.01	0.01	
TBARS (mg MA/kg)	5	0.59 <sup>bC</sup>	0.50 <sup>dC</sup>	0.58 <sup>cC</sup>	1.08 <sup>aB</sup>	0.01
	15	0.88 <sup>bB</sup>	0.55 <sup>dB</sup>	0.63 <sup>cB</sup>	1.08 <sup>aB</sup>	0.03
	30	0.96 <sup>bA</sup>	0.79 <sup>dA</sup>	0.89 <sup>cA</sup>	1.41 <sup>aA</sup>	0.01
	SEM	0.02	0.01	0.03	0.01	

Means in a row with different letters (a-d) are significantly different ( $P < 0.05$ ).

Means in a column with different letters (A-C) are significantly different ( $P < 0.05$ ).

MLE, ethanolic extract of *Moringa stenopetala* leaves; SEM, standard error of the mean; TBARS, thiobarbituric acid reactive; MA, malonaldehyde.

<sup>1)</sup>Control, without any preservative; MLE1 (0.10%); MLE2 (0.05%); PS, potassium sorbate (0.20%).

PS had higher TBARS values than the control and MLE treatments throughout the storage study. This may be due to the activity of PS as a catalyst in the oxidation process. Love and Pearson (1971) documented that sodium chloride accelerates triglyceride oxidation though the salt catalysis mechanism is not fully known. A concentration-dependent increase in retarding lipid oxidation was noticed with MLE addition. As the concentration of MLE increased from 0.05% to 0.10% in treated the patties, there was progressive retardation in the oxidation of samples. On the final storage day, day 30, the TBARS values in the control, MLE1, MLE2, and PS samples were 0.96, 0.79, 0.89, and 1.41 mg MA/kg meat, respectively. According to O'Byrne et al. (2002), the inhibition of lipid peroxidation in moringa leaf powder may be due to its polyphenols content that contributes a significant role in the absorption and neutralization of the free radicals. In a previous study, Das et al. (2012) documented that the incorporation of MOL water extract at 0.10% concentration was enough to retard lipid oxidation of cooked goat meat patties for a longer period than the commonly used synthetic antioxidant BHT, as estimated by TBARS value during refrigerated storage. Inhibition of lipid oxidation was succeeded in this study using ethanolic extract of *M. stenopetala* leaves at a lower concentration of 0.05% in vacuum-packed pork patties at the same storage condition. In comparison to previous results, the effective antioxidant concentration was decreased by half, from 0.10 to 0.05%. The duration of storage showed a significant ( $P < 0.05$ ) influence in the TBARS values of all experimental samples. As the storage time extended from 5 to 30 days, the TBARS values increased in the same samples from 0.59 to 0.96, 0.50 to 0.79, 0.58 to 0.89, and 1.08 to 1.41 mg MA/kg sample for control, MLE1, MLE2,

and PS, respectively. The change in TBARS was very low in MLE treated samples and persisted up to 30 days. According to Trindade et al. (2009), TBARS values of foods that are higher than 2.00 mg of MA/kg sample are considered as harmful to the consumer's health. The amounts of MA/kg samples in the present study were all lower than 2.00 and vary between 0.05 and 1.41 mg at 30 days storage, which is considered a safe limit. Generally, the presented results confirm the existence of antioxidant activity of MLE as applied in the pork patties with 0.10% and 0.05% concentrations during refrigeration storage.

The effect of incorporation of MLE on microbial quality of patties during the storage time is presented in Table 2. Patties incorporated with MLE had a significantly lower ( $P < 0.05$ ) total plate count than the control, without added preservative, but the counts were higher as compared to the patties added with the commercial inorganic preservative, PS, across all the storage period. The growth of microorganisms in pork patties reduced concurrently with an increase in MLE concentration during storage. The existence of phytochemical compounds such as polyphenols and flavonoids and other peptides and proteins may contribute to this antimicrobial action. Fahey (2005) reported that leaves of the moringa plant contain an appreciable amount of low-molecular-weight proteins and peptides, which are recognized to have antimicrobial properties (Fahey, 2005). Similarly, previous studies attested that the addition of moringa leaf extracts reduced the total plate count during the storage of various meat products (Shah et al., 2015; Falowo et al., 2016). Moreover, Al-Juhaimi et al. (2015) demonstrated that the incorporation of moringa seed powder into beef patties substantially reduced the aerobic total plate count during 3 weeks of storage. In all patties for the current study,

**Table 2.** Microbiological characteristics of patties (log CFU/g) incorporated with MLE during the storage time

Attributes	Days	Treatments of patties <sup>1)</sup>				SEM
		Control	MLE1	MLE2	PS	
TPC (log CFU/g)	5	— <sup>C</sup>	— <sup>C</sup>	— <sup>C</sup>	— <sup>C</sup>	0.01
	15	5.66 <sup>aB</sup>	5.09 <sup>cB</sup>	5.50 <sup>bB</sup>	4.90 <sup>dB</sup>	0.08
	30	7.28 <sup>aA</sup>	6.66 <sup>bA</sup>	6.90 <sup>bA</sup>	5.82 <sup>cA</sup>	0.15
	SEM	0.08	0.17	0.18	0.09	
YM <sup>2)</sup> (log CFU/g)	5	—	—	—	—	ND
	15	—	—	—	—	ND
	30	—	—	—	—	ND
	SEM	ND	ND	ND	ND	

Means in a row with different letters (a-d) are significantly different ( $P < 0.05$ ).

Means in a column with different letters (A-C) are significantly different ( $P < 0.05$ ).

MLE, ethanolic extract of *Moringa stenopetala* leaves; TPC, total plate count; YM, yeast and mold; SEM, standard error of the mean.

ND, not determined.

<sup>1)</sup>Treatments are as indicated in Table 1.

<sup>2)</sup>ANOVA was not done because the counts were  $< 10$  CFU/g across the storage days.

substantial increases ( $P<0.05$ ) in the TPC were exhibited in control and treated samples with an increase in storage period, but it concomitantly ( $P<0.05$ ) declined with an increase in MLE concentration and the use of PS. There was no TPC in all samples at 5 days of storage. However, the TPCs were increased to 7.28, 6.66, 6.90, and 5.82 logs CFU/g for the control, MLE1, MLE2, and PS samples, respectively at 30 days storage. All the patties in the present study did not have yeast and mold count across the storage days. This might be due to the combined effect of cooking, vacuum packaging, and storage temperature followed in the present study that inhibited the growth of yeast and mold.

Color is one of the main important sensory attributes, which governs the extent of product acceptability. The characteristics for instrumental color values of pork patties incorporated with MLE during storage time are presented in Table 3. There is a significant difference ( $P<0.05$ ) in  $L^*$  values among the groups of patties. As compared to the control, significant lower  $L^*$  values were exhibited in both groups of patties treated with 0.05 and 0.10% concentration of MLE. Similarly, Naveena et al. (2008) reported that incorporation of pomegranate rind powder extract in chicken patties resulted in the reduction of  $L^*$  value, whereas Carpenter et al. (2007) did not exhibit any variation in  $L^*$  value after the addition of grape seed extract or bearberry in raw pork patties. There was no significant variation in the  $L^*$  value of the control and MLE treated samples as the duration of storage time extended from 5 to 30 days. However, the value tended to increase as the storage time progressed from 5 to 15 days in the control, MLE1 and PS. Particularly, the stor-

age time showed a substantial effect in the PS treated sample. The increasing trend in  $L^*$  value for treatments between 5 and 15 days is probably due to a surface discoloration and progressively increase in metmyoglobin (MMb) content during storage (Jeremiah and Gibson, 2001; Muthukumar et al., 2014). The higher lightness could be also attributed to the dilution of the color by incorporated material into the patties formulation (Eshag Osman et al., 2021). Similarly, the increase  $L^*$  value during storage was reported in earlier studies of Eshag Osman et al. (2021) and Jo et al. (2015).  $a^*$  value is the most indispensable criterion for the assessment of oxidation, and the decrement of redness in meat is an important indicator of oxidation (Ergezer et al., 2018). In the present study, the addition of MLE, both at 0.10 and 0.05% concentration, presented significantly higher ( $P<0.05$ )  $a^*$  values than the control across the storage days, and the values became comparable with PS treated samples as days of storage extended (Table 3). The red color of the meat is principally due to the myoglobin content. Myoglobin is highly vulnerable to oxidation into MMb as the result of lipid oxidation and microbial spoilage (Muthukumar et al., 2014). In all treatment groups,  $a^*$  value showed a significant decrease ( $P<0.05$ ) due to prolonged storage time. The change in color from red to brown because of metmyoglobin generation may be attributed to a decrease in  $a^*$  value of patties during storage. In the previous study by Carpenter et al. (2007), progressive decreases in the redness value of pork patties were reported during refrigerated display conditions. The addition of MLE was exhibited to affect the  $b^*$  value of pork patties. As the duration of storage increased from 5

**Table 3.** Color values of patties incorporated with MLE during the storage time

Attributes	Days	Treatments of patties <sup>1)</sup>				SEM
		Control	MLE1	MLE2	PS	
$L^*$	5	65.54 <sup>a</sup>	62.32 <sup>b</sup>	62.47 <sup>b</sup>	60.57 <sup>bB</sup>	1.66
	15	67.20 <sup>a</sup>	64.14 <sup>b</sup>	62.40 <sup>b</sup>	63.43 <sup>bA</sup>	1.75
	30	66.68 <sup>a</sup>	62.25 <sup>b</sup>	61.16 <sup>c</sup>	62.42 <sup>bAB</sup>	0.70
	SEM	1.41	1.60	1.14	1.60	
$a^*$	5	11.59 <sup>cA</sup>	12.87 <sup>b</sup>	12.80 <sup>bA</sup>	14.10 <sup>aA</sup>	0.65
	15	11.24 <sup>bA</sup>	12.62 <sup>a</sup>	12.70 <sup>aA</sup>	12.98 <sup>aB</sup>	0.56
	30	10.99 <sup>cB</sup>	12.78 <sup>a</sup>	11.53 <sup>bB</sup>	12.81 <sup>aB</sup>	0.37
	SEM	0.79	0.56	0.32	0.38	
$b^*$	5	8.84 <sup>ab</sup>	9.27 <sup>aA</sup>	7.97 <sup>bB</sup>	8.16 <sup>abA</sup>	0.83
	15	8.16 <sup>ab</sup>	8.81 <sup>aAB</sup>	8.15 <sup>abB</sup>	7.27 <sup>bB</sup>	0.81
	30	9.09 <sup>a</sup>	7.82 <sup>dB</sup>	8.62 <sup>bA</sup>	8.36 <sup>cA</sup>	0.19
	SEM	0.86	0.83	0.23	0.59	

Means in a row with different letters (a-d) are significantly different ( $P<0.05$ ).

Means in a column with different letters (A-C) are significantly different ( $P<0.05$ ).

MLE, ethanolic extract of *Moringa stenopetala* leaves; SEM, standard error of the mean.

<sup>1)</sup>Treatments are as indicated in Table 1.

to 30 days, the variation in  $b^*$  value of treatment groups was pronounced, and the control group had a significantly higher ( $P < 0.05$ )  $b^*$  value as compared to both MLE and PS treated samples (Table 3). According to Collins and Huey (2014), noticeable rancidity in meat is generally accompanied by changes in color for the oxidation reaction of the fat, from white to yellow. The present finding agrees with Muthukumar et al. (2014) who noticed a decrease in  $b^*$  value in raw and cooked pork patties as a result of MOL extract addition as a natural antioxidant. Moreover, Rojas and Brewer (2008) observed a reduction in the  $b^*$  value of beef patties added with natural antioxidants. However, Carpenter et al. (2007) have not detected any change in the  $b^*$  value of raw pork patties after the incorporation of bearberry or grape seed extract.

Measuring sensory quality attributes is the most important concept in the prediction of oxidative stability, shelf life, and market acceptability of processed meat products. Descriptive sensory analysis was employed to compare treatments in six different sensory quality attributes including color, aroma, texture, flavor, juiciness, and overall acceptability. In all sensory attributes examined in the current study, the addition of 0.10 and 0.05% MLE concentration did not have any significant variation ( $P <$

0.05) from the control and PS treated samples during the storage study (Table 4). Texture, flavor, juiciness, and overall acceptability attributes of all treatment samples have not been affected by storage time ( $P < 0.05$ ). However, the storage time had a substantial effect ( $P < 0.05$ ) in color and aroma only in PS and 0.10% MLE treated samples, respectively. The increase in aroma characteristics of MLE1 samples for the prolonged storage time may be associated with evolution of volatile aroma compounds, which are endowed by moringa plants. Mukunzi et al. (2011) reported a total of ninety-three volatiles from moringa leaves obtained from Rwanda and China, and the most abundant compounds were acetic acid, 3,3-dimethyl-cyclohexanol and dihydroactinidolide. Ali et al. (2015) identified acetic acid and 10 other aroma volatiles in guava whey beverage fortified with moringa leaf extract and the most abundant compounds were 2-hexyldecanoic acid, dodecanoic acid, and docanoic acid, 2-hexyl. The color characteristics in the PS treated sample significantly decreased at the end of the storage period in the panel's judgment. This may be associated with salt-initiated oxidative rancidity in PS samples which are observed in TBARS development (Table 1) of this treatment as the storage time extended. Unlike other treated samples and control, the sample treated with MLE1

**Table 4.** Sensory quality of patties incorporated with MLE during the storage time

Attributes	Days	Treatments of patties <sup>1)</sup>				SEM
		Control	MLE1	MLE2	PS	
Color	5	3.29	3.57	3.43	4.14 <sup>A</sup>	0.64
	15	3.14	3.11	2.93	3.29 <sup>B</sup>	0.85
	30	3.43	2.71	3.29	3.14 <sup>B</sup>	0.56
Aroma	5	3.29	2.43 <sup>B</sup>	3.07	3.00	0.72
	15	3.14	2.36 <sup>B</sup>	2.36	2.71	0.61
	30	3.43	3.14 <sup>A</sup>	3.21	3.29	0.50
Texture	5	4.00	3.43	3.43	3.71	0.79
	15	3.57	3.14	2.97	3.57	0.83
	30	3.29	3.17	2.86	3.57	0.79
Flavor	5	3.43	3.36	3.26	3.57	0.75
	15	3.29	3.14	2.56	3.29	1.09
	30	3.43	2.93	2.86	3.07	0.98
Juiciness	5	3.14	3.29	3.00	3.14	0.85
	15	3.00	2.43	2.14	2.43	0.96
	30	3.43	3.26	2.71	3.21	0.88
Overall acceptability	5	3.29	3.07	3.30	3.50	0.64
	15	3.57	3.03	3.36	3.14	0.78
	30	3.43	2.96	3.00	3.14	0.44

Means in a column with different letters (A-C) are significantly different ( $P < 0.05$ ).

MLE, ethanolic extract of *Moringa stenopetala* leaves; SEM, standard error of the mean.

<sup>1)</sup>Treatments are as indicated in Table 1.

showed an increased aroma development towards the final storage period as noticed by the panel. However, this treatment showed a decreased color value, even if the change is not significant, for the extended storage time that may not encourage the addition of a MLE extract above 0.10%.

In conclusion, incorporation of MLE (0.10 and 0.05% concentration) delayed the onset of oxidative rancidity and reduced microbial growth in pork patties during storage without any effect on the sensory properties and overall acceptability as perceived by the panel. Therefore, the use of MLE can help in extending the shelf life of pork patties to satisfy both the current consumer's requirements for organic, healthy, and safe food ingredients and can add to market value of MLE as an organic preservative.

## ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2017R1A2B201277).

## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

## REFERENCES

- Abiyu A, Yan D, Girma A, Song X, Wang H. Wastewater treatment potential of *Moringa stenopetala* over *Moringa olifera* as a natural coagulant, antimicrobial agent and heavy metal removals. *Cogent Environ Sci*. 2018. 4:1433507. <https://doi.org/10.1080/23311843.2018.1433507>
- Al-Juhaimi F, Ghafoor K, Hawashin MD, Alsawmahi ON, Babiker EE. Effects of different levels of moringa (*Moringa oleifera*) seed flour on quality attributes of beef burgers. *CyTA-J Food*. 2015. 2015:1034784. <https://doi.org/10.1080/19476337.2015.1034784>
- Ali AO, Elreffaei WHM, Elkarmany AM, Elsheek FMA. Quality of guava whey beverage fortified with *Moringa oleifera* leaves extract. *Life Sci J*. 2015. 12:113-122.
- Biswas AK, Keshri RC, Bisht GS. Effect of enrobing and antioxidants on quality characteristics of precooked pork patties under chilled and frozen storage conditions. *Meat Sci*. 2004. 66: 733-741.
- Carpenter R, O'Grady MN, O'Callaghan YC, O'Brien NM, Kerry JP. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Sci*. 2007. 76: 604-610.
- Collins DS, Huey RJ. Meat decomposition and spoilage. In: Gracey's Meat Hygiene, 11th ed. John Wiley & Sons, Inc., London, UK. 2014. p 271-276.
- Das AK, Rajkumar V, Verma AK, Swarup D. *Moringa oleifera* leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties. *Int J Food Sci Technol*. 2012. 47: 585-591.
- Decker EA, Chan WKM, Livisay SA, Butterfield DA, Faustman C. Interactions between carnosine and the different redox states of myoglobin. *J Food Sci*. 1995. 60:1201-1204.
- Dev SRS, Geetha P, Orsat V, Gariépy Y, Raghavan GSV. Effects of microwave-assisted hot air drying and conventional hot air drying on the drying kinetics, color, rehydration, and volatiles of *Moringa oleifera*. *Drying Technol*. 2011. 29:1452-1458.
- Devatkal SK, Thorat P, Manjunatha M. Effect of vacuum packaging and pomegranate peel extract on quality aspects of ground goat meat and nuggets. *J Food Sci Technol*. 2014. 51:2685-2691.
- Drosinos EH, Mataragas M, Xiraphi N, Moschonas G, Gaitis F, Metaxopoulos J. Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Sci*. 2005. 69:307-317.
- Ergezer H, Kaya Hİ, Şimşek Ö. Antioxidant and antimicrobial potential of artichoke (*Cynara scolymus* L.) extract in beef patties. *Czech J Food Sci*. 2018. 36:154-162.
- Eshag Osman MF, Mohamed AA, Alamri MS, Ahmed IAM, Hussain S, Ibraheem MI, et al. Quality characteristics of beef patties prepared with octenyl-succinylated (Osan) starch. *Foods*. 2021. 10:1157. <https://doi.org/10.3390/foods10061157>
- Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*. 2001. 56:5-51.
- Fahey JW. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees Life J*. 2005. 1:5. <https://doi.org/10.1201/9781420039078.ch12>
- Falowo AB, Fayemi PO, Muchenje V. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: a review. *Food Res Int*. 2014. 64:171-181.
- Falowo AB, Muchenje V, Hugo CJ, Charimba G. *In vitro* antimicrobial activities of *Bidens pilosa* and *Moringa oleifera* leaf extracts and their effects on ground beef quality during cold storage. *CyTA-J Food*. 2016. 14:541-546.
- Gill CO. Meat spoilage and evaluation of the potential storage life of fresh meat. *J Food Prot*. 1983. 46:444-452.
- Hazra S, Biswas S, Bhattacharyya D, Das SK, Khan A. Quality of cooked ground buffalo meat treated with the crude extracts of *Moringa oleifera* (Lam.) leaves. *J Food Sci Technol*. 2012. 49: 240-245.
- Jahn SAA. The traditional domestication of a multipurpose tree *Moringa stenopetala* (Bak.f.) Cuf. in the Ethiopian Rift Valley. *Ambio*. 1991. 20:244-247.
- Jayathilakan K, Sharma GK, Radhakrishna K, Bawa AS. Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. *Food Chem*. 2007. 105:908-916.
- Jeremiah LE, Gibson LL. The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Res Int*. 2001. 34:815-826.
- Jo YJ, Kwon YJ, Min SG, Choi MJ. Changes in quality characteristics of pork patties containing multilayered fish oil emulsion during refrigerated storage. *Korean J Food Sci Anim Resour*. 2015. 35:71-79.
- Juntachote T, Berghofer E, Siebenhandl, S, Bauer F. The antioxidative properties of Holy basil and Galangal in cooked ground pork. *Meat Sci*. 2006. 72:446-456.
- Kanner J. Oxidative processes in meat and meat products: quality implications. *Meat Sci*. 1994. 36:169-189.
- Kim SJ, Cho AR, Han J. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*. 2013. 29:112-120.
- Love JD, Pearson AM. Lipid oxidation in meat and meat products – a review. *J Am Oil Chem Soc*. 1971. 48:547-549.



- Mahmoud MH, Abou-Arab AA, Abu-Salem FM. Quality characteristics of beef burger as influenced by different levels of orange peel powder. *Am J Food Technol.* 2017. 12:262-270.
- Mukunzi D, Nsor-Atindana J, Xiaoming Z, Gahungu A, Karangwa E, Mukamurezi G. Comparison of volatile profile of *Moringa oleifera* leaves from Rwanda and China using HS-SPME. *Pak J Nutr.* 2011. 10:602-608.
- Muthukumar M, Naveena BM, Vaithyanathan S, Sen AR, Sureshkumar K. Effect of incorporation of *Moringa oleifera* leaves extract on quality of ground pork patties. *J Food Sci Technol.* 2014. 51:3172-3180.
- Najeeb AP, Mandal PK, Pal UK. Efficacy of leaves (drumstick, mint and curry leaves) powder as natural preservatives in restructured chicken block. *J Food Sci Technol.* 2015. 52:3129-3133.
- Naveena BM, Sen AR, Vaithyanathan S, Babji Y, Kondaiah N. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Sci.* 2008. 80:1304-1308.
- Núñez de Gonzalez MT, Hafley BS, Boleman RM, Miller RK, Rhee KS, Keeton JT. Antioxidant properties of plum concentrates and powder in precooked roast beef to reduce lipid oxidation. *Meat Sci.* 2008. 80:997-1004.
- O'Byrne DJ, Devaraj S, Grundy SM, Jialal I. Comparison of the antioxidant effects of Concord grape juice flavonoids  $\alpha$ -tocopherol on markers of oxidative stress in healthy adults. *Am J Clin Nutr.* 2002. 76:1367-1374.
- Padayachee B, Baijnath H. An overview of the medicinal importance of Moringaceae. *J Med Plants Res.* 2012. 6:5831-5839.
- Pikul J, Leszczynski DE, Kummerow FA. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J Agric Food Chem.* 1989. 37:1309-1313.
- Rojas MC, Brewer MS. Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. *J Food Qual.* 2008. 31:173-188.
- Shah MA, Bosco SJD, Mir SA. Effect of *Moringa oleifera* leaf extract on the physicochemical properties of modified atmosphere packaged raw beef. *Food Packag Shelf Life.* 2015. 3:31-38.
- Shah MA, Bosco SJD, Mir SA. Plant extracts as natural antioxidants in meat and meat products. *Meat Sci.* 2014. 98:21-33.
- Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agric Food Chem.* 2003. 51:2144-2155.
- Tang S, Kerry JP, Sheehan D, Buckley DJ, Morrissey PA. Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. *Food Res Int.* 2001. 34:651-657.
- Trindade RA, Mancini-Filho J, Villavicencio ALCH. Effects of natural antioxidants on the lipid profile of electron beam-irradiated beef burgers. *Eur J Lipid Sci Technol.* 2009. 111:1161-1168.